

## Phenotypic Detection of Prevalence of Carbapenemase Producing Enterobacteriaceae Isolates in a Tertiary Care Hospital, RIMS, Ranchi.

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**Abstract –Introduction** – The Modified Hodge test (MHT) detects carbapenemase production in isolates of Enterobacteriaceae. It is detected when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922) towards a carbapenem disk producing a characteristic cloverleaf-like indentation [1]. **Aims and objectives** – To evaluate the epidemiology of carbapenemase producing Enterobacteriaceae isolates in a tertiary care hospital, RIMS, Ranchi. **Methodology** – A total of 678 isolates belonging to the family Enterobacteriaceae were obtained over a period of 6 months. Amongst these, 201 showed intermediate or resistant zone of inhibition for Meropenem (10µg) disk, which were selected for further processing by MHT. **Results and Discussion** – 23.15% (157) of the total (678) and 78.10% of those which showed intermediate or resistant zone of inhibition, gave positive results for MHT. **Conclusion** – Enterobacteriaceae members are among the most common and easily transferable species causing nosocomial infections [2]. Hence, it will be helpful in preventing the spread of resistant strains, if MHT is incorporated in routine practice.

**Keywords** – carbapenem resistance, carbapenemases, Modified Hodge test, Enterobacteriaceae, cloverleaf like indentation.

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Date of Submission: 01-02-2019

Date of acceptance: 18-02-2019

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### I. Introduction

At least eight distinctive mechanisms of antibiotic resistance have been described in bacteria. One among them is Enzymatic inhibition. Resistance to  $\beta$ -lactam antibiotics which include penicillins, cephalosporins, monobactams and carbapenems occurs primarily through production of  $\beta$ -lactamases.  $\beta$ -lactamases are enzymes that inactivate these antibiotics by splitting the amide bond of the  $\beta$ -lactam ring.  $\beta$ -lactamases have been classified by Ambler [3] into 4 classes A, B, C and D. Alternatively, Bush-Jacoby-Medeiros [4] system classifies the enzyme into several functional groups 1, 2a, 2b, 2be, 2br, 2c, 2d, 2e, 2f, 3 and 4.

Classes A, B, D of Ambler classification and Groups 2f, 3 of Bush-Jacoby-Medeiros system comprise Carbapenemases. Carbapenemases confer the largest antibiotic resistance spectrum because they hydrolyze not only carbapenems but also broad spectrum penicillins, oxymino-cephalosporins and cephamycins.

In the past few years, carbapenems have been regarded as the treatment of choice for infections caused by resistant strains of gram negative bacteria [5]. But, unfortunately many recent studies have reported emergence of carbapenem resistance among Enterobacteriaceae.

### II. Aims And Objectives

This study was aimed at detecting the prevalence of carbapenemase activity in members of Enterobacteriaceae group that are isolated from various samples processed by using the phenotypic screening method of Modified Hodge test.

### III. Materials And Methods

This study was done at Department of Microbiology of a tertiary care hospital, RIMS, Ranchi, Jharkhand, India between January 2017 to June 2017.

The urine samples were inoculated in CLED agar while all other samples were inoculated on both MacConkey and Blood agar plates. All the plates were incubated overnight at 37°C. Next morning the isolates were identified by their cultural characteristics, colony morphology, Gram's staining and biochemical properties. All those isolates which belonged to the Family Enterobacteriaceae were separated and kept for further processing.

A total of 678 gram negative bacilli belonging to Enterobacteriaceae, isolated from clinical samples of urine, pus, sputum, blood and different body fluids were included in the study.

All 678 isolates were put for susceptibility testing against 10 $\mu$ g disk of Meropenem and the ones which showed zone diameter  $\leq$  22mm [7] were selected for MHT.

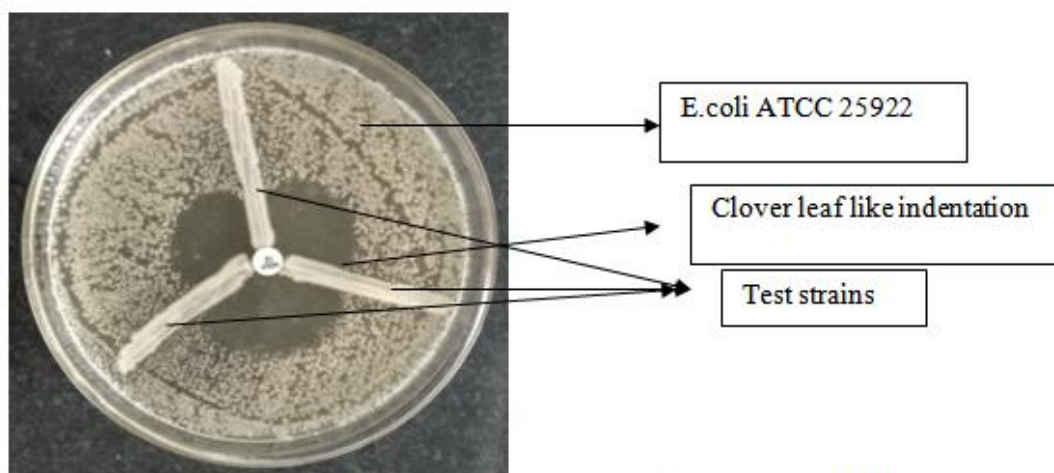
A working culture was made from the stored E.coli ATCC 25922. Colonies of E.coli ATCC were used to make 0.5 McFarland suspension. Later this was converted into 1:10 dilution [8].

The diluted suspension was streaked as lawn culture on to a Mueller Hinton Agar. The plate was allowed to dry for a 3-10 minutes and then, a 10 $\mu$ g Meropenem disk was placed in the centre of the plate. 3-5 colonies [9] of the separated test organisms are streaked in straight lines from the edge of the disk to edge of the plate.

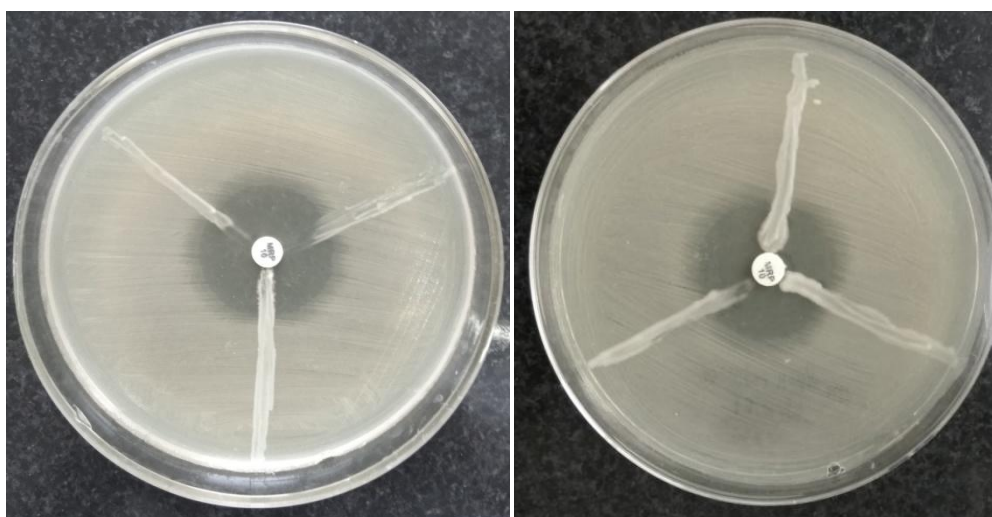
The plates were incubated overnight at 37 $^{\circ}$ C for 18-24hrs.

#### IV. Results

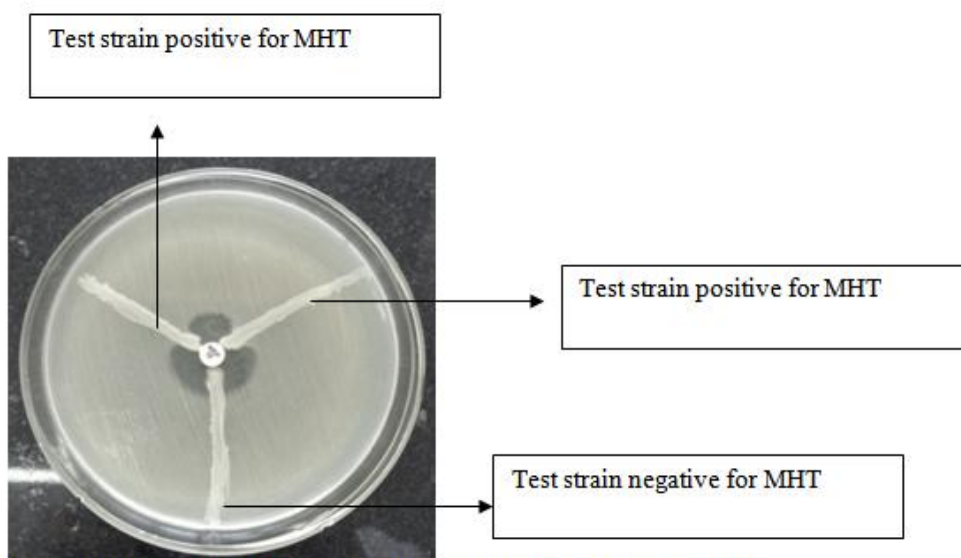
MHT positive test showed a clover leaf like indentation of the E. coli ATCC 25922 [6] growing along the test organism growth streak within the disk diffusion zone.



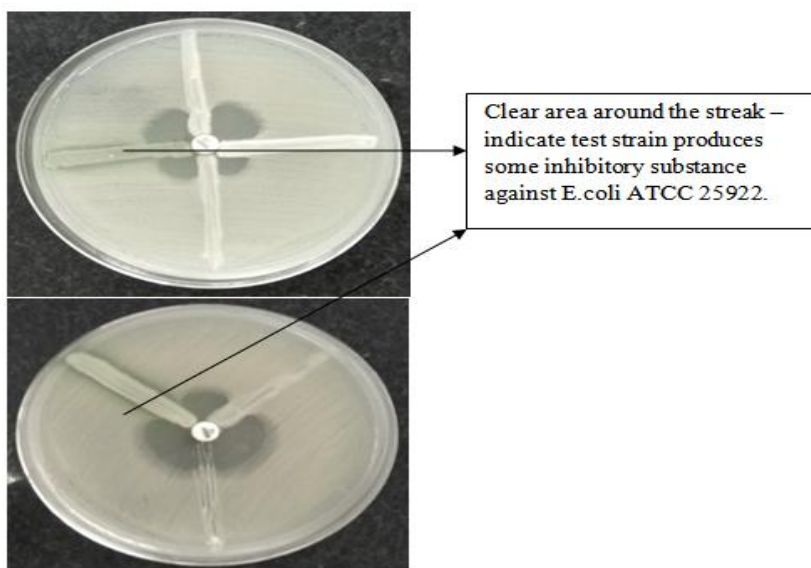
**Figure 1: Plate showing all the 3 isolates positive for MHT.**



**Figure 2,3: Plates showing all the 3 isolates negative for MHT.**

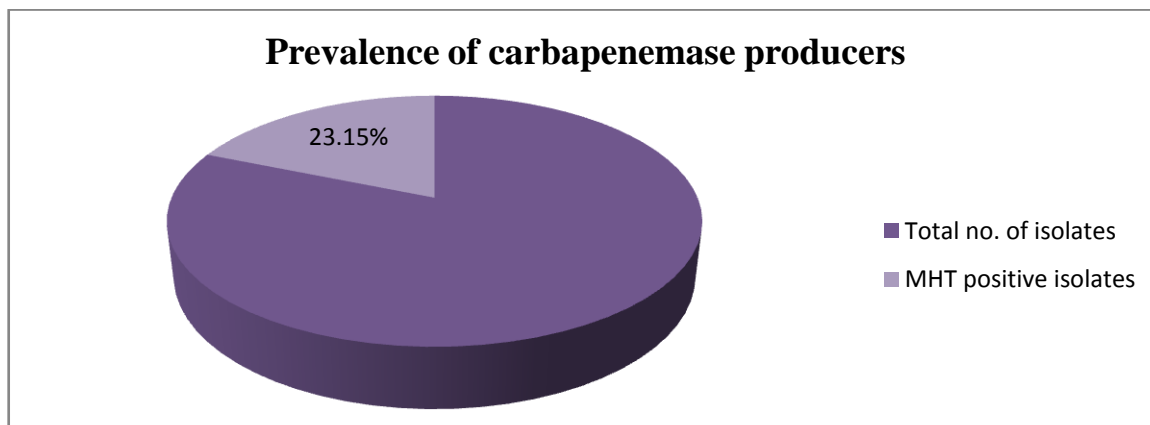


**Figure 4: Plate showing 2 isolates positive while 1 isolate negative for MHT.**



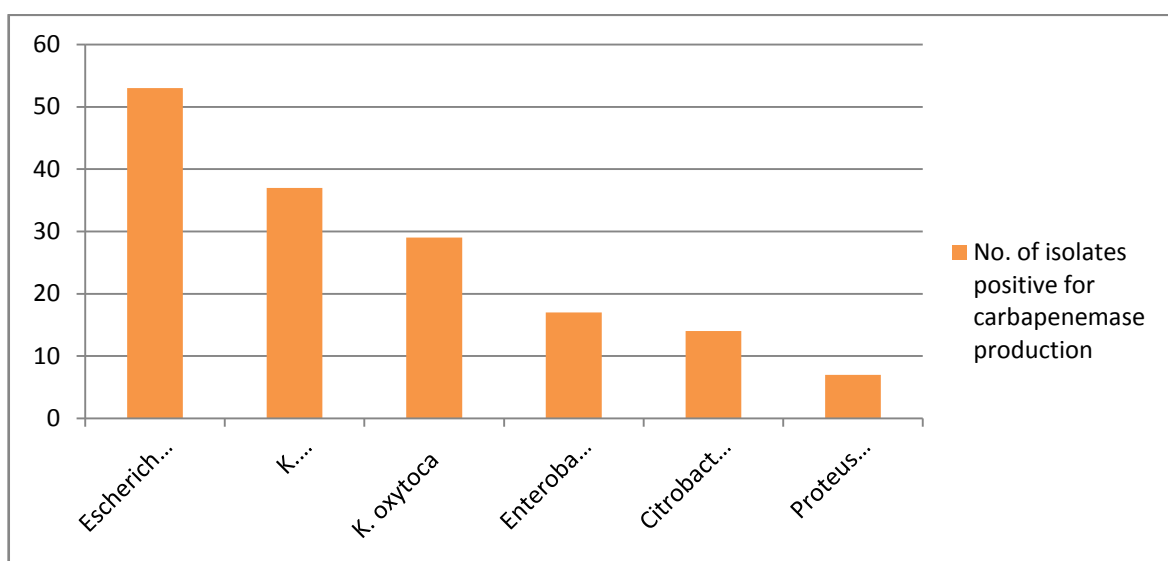
**Figure 5,6: Plates showing indeterminate results.**

Out of 678 isolates which belonged to the family Enterobacteriaceae, 201 were found to produce either resistant or intermediate zones of inhibition for 10µg disk of meropenem. Amongst these 201 isolates, 157 were positive for Modified hodge test.



**Figure 7: Pie chart depicting prevalence of carbapenemase producers among Enterobacteriaceae**

The different bacteria constituting the MHT positive spectrum were Escherichia coli (33.75%), Klebsiella pneumoniae (23.56%), Klebsiella oxytoca(18.47%), Enterobacter spp.(10.82%), Citrobacter spp.(8.9%) and Proteus spp. (4.45%).



**Figure 8: Bargraph depicting distribution of carbapenemase producers among various species isolated.**

SAMPLES	No. Of Enterobacteriaceae isolates	No. and percentage of isolates which showed Positive modified hodge test.
URINE	301	88
PUS	102	29
BODY FLUIDS	99	8
SPUTUM	89	19
BLOOD	87	13
<b>TOTAL</b>	<b>678</b>	<b>157</b>

**Table no. 1 –No. of Enterobacteriaceae isolates isolated and the no. of isolates found positive in MHT, among different sample types.**

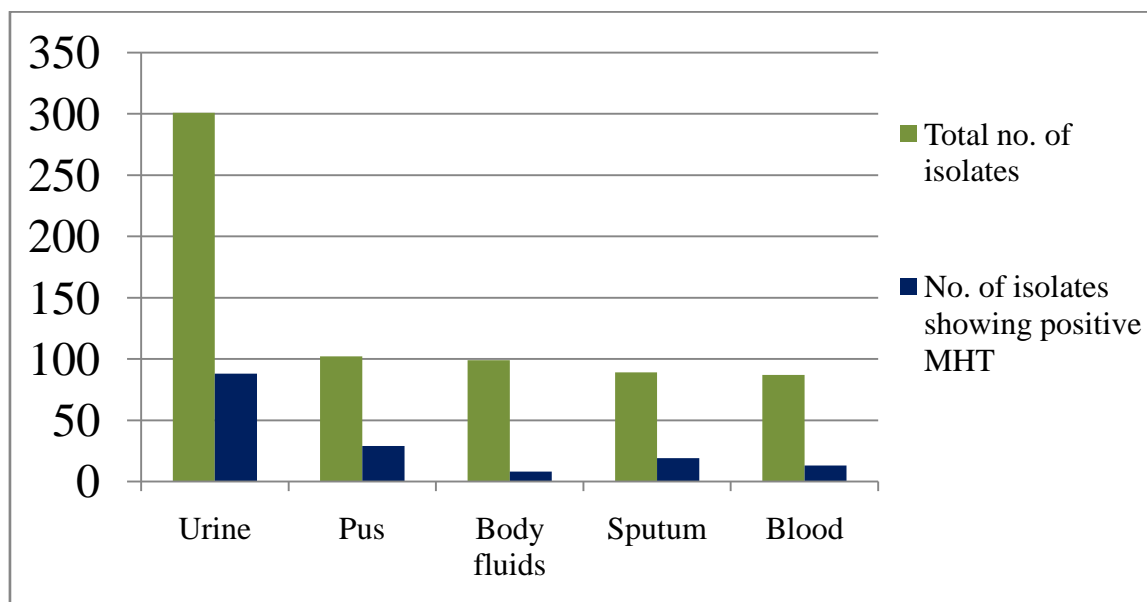


Figure 9: Bargraph depicting fraction of isolates which were carbapenemase producers among various sample types.

Majority of the carbapenemase producing isolates of Enterobacteriaceae group were obtained from samples collected from patients admitted to ICUs (98) followed by the inpatient wards (37) and OPDs (22).

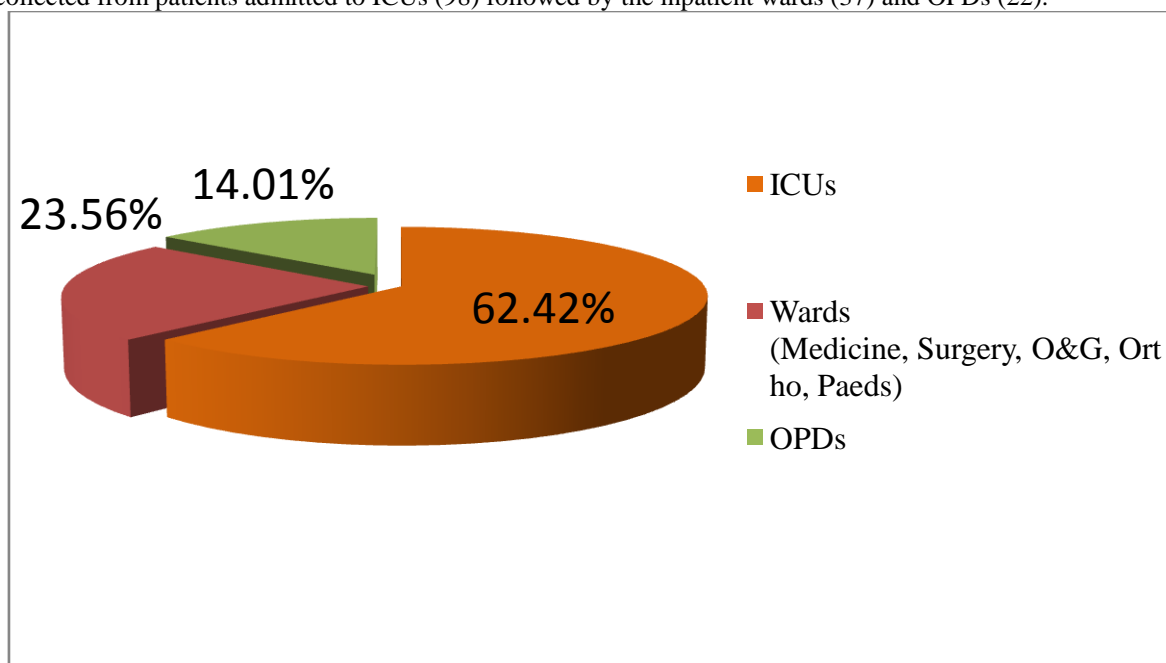


Figure 10 : Pie chart depicting the percentage of isolates found positive for carbapenemase production in samples obtained from different sections of the hospital.

### V. Discussion

In the present study, 23.15% of the isolates were positive for carbapenemase production by MHT which was in concordance with the results (25.5%) in a study conducted by Takayama et al [6]. In the study done by Bora et al [17], all the isolates which had reduced susceptibility to meropenem or ertapenem showed positive MHT, however, in our study 78.10% isolates showed positive MHT, which was not much in concordance. Bora et al reported 53.56% positive isolates to be coming from ICUs while the percentage obtained in our study was slightly higher (62.42%).

The Modified Hodge test as recommended by CLSI, is a phenotypic screening test with a high sensitivity and specificity [12] in detecting KPC type carbapenemase producers. It has a 100% negative predictive value. However, false positive results have been reported, when compared with PCR results, which

may be due to AmpC enzymes [6], ESBLs [11] or loss of porins [13]. But unavailability and high cost of genotyping, indirectly makes the phenotypic methods more easily and widely used ones.

The limitations of MHT are lack of specificity and the delay in obtaining results. This method cannot determine the class of carbapenemase [1].

## VI. Conclusion

Many patients are prescribed carbapenems even without testing the pathogens for carbapenemase production [5]. Besides causing treatment failure, it would lead to the development of more number of resistant strains. These resistant strains can take up the hospital environment where the sensitive strains are removed due to use of antimicrobials. The resistant strains start to flourish in the hospital environment and cause hospital acquired infections among the indoor patients. This would ultimately affect the country's economy adversely because the amount of expenditure in the health sector, will have to be enormously increased, so as to treat all the nosocomial infections as well.

Hence, to avoid these, Modified Hodge test is a simple screening test that can be inculcated in the routine practice to screen for carbapenemase production.

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Manoj Kumar" Phenotypic Detection of Prevalence of Carbapenemase Producing Enterobacteriaceae Isolates in a Tertiary Care Hospital, Rims, Ranchi."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 2, 2019, pp 19-24.